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In re application of:	Examiner: Unassigned
Juha Punnonen et al.	Art Unit: Unknown
Application No.: 09/760,388	
Filed: January 10, 2001	PRELIMINARY AMENDMENT
For: MONOCYTE-DERIVED DENDRITIC CELL SUBSETS	

Assistant Commissioner for Patents
Washington, D.C. 20231

Prior to examination of the above-identified Application, please amend the Application as follows.

The following correspondence is enclosed herewith:

1) transmittal form
2) marked-up copy of the claims
3) courtesy copy of the claims as amended
4) marked copy of the relevant portions of the specification
5) marked copies of Figure 2 and Figure 3
6) substitute copies of corrected Figure 2 and Figure 3
7) Information Disclosure Statement
8) PTO-1449 Form
9) Copies of Cited References
10) receipt acknowledgement postcard

IN THE FIGURES:

Please substitute appended Figure 2 and Figure 3 for Figures 2 and 3 as filed. A corrected version showing changes in red ink is also enclosed. Substitute figures are provided merely to correct inadvertent mislabeling of the Figures, and no new matter is presented by these amendments.

IN THE SPECIFICATION:

Please amend the specification by substituting the following paragraphs as indicated below, without prejudice to subsequent renewal of the specification in its original form. These amendments introduce no new matter and are merely provided to correct inadvertent typographical errors, as noted in more detail below.

Page 1, line 17 through 20:

This application claims priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/175,552, filed on January 11, 2000, and 60/181,957, filed on February 10, 2000, the disclosure of each of which is incorporated herein by reference in its entirety for all purposes.

Page 6, line 10 through 12:

Figure 6. A series of bar graphs illustrating T cell differentiation in the presence of mDC1 and mDC2: (A) IFN-γ production; (B) IL-5 (open bars) and IL-13 (filled bars) production; (C) ratio of IFN-γ/IL-5 production; and (D) ratio of IFN-γ/IL-13 production.

Page 7, lines 10 through 13:

In contrast with conventional monocyte-derived dendritic cells which strongly favor Th1 differentiation, the unique monocyte-derived dendritic cells of the present invention favor differentiation of Th0/Th2 cells when co-cultured with purified human T cells.

Page 13, lines 22 through 37:

Pathogens and diseased cells, e.g., tumor, necrotic, or apoptotic cells, express a variety of antigens implicated in the cell-mediated immune response against the target cell. It is expected that one of ordinary skill in the art is familiar with the identity of many such antigens.

T cells recognizing such epitopes are stimulated to proliferate in response to antigen presenting cells, such as dendritic cells, including the dendritic cells of the present invention, which display an antigen on a MHC molecule. Examples of antigens include tumor derived antigens, e.g., prostate specific antigen (PSA), colon cancer antigens (e.g., CEA), breast cancer antigens (e.g., HER-2), leukemia antigens, and melanoma antigens (e.g., MAGE-1, MART-1); antigens to lung, colorectal, brain, pancreatic cancers; antigens to renal cell carcinoma, lung, colorectal, pancreatic B-cell lymphoma, multiple myeloma, prostate carcinomas, sarcomas, and neuroblastomas; viral antigens, e.g., hepatitis B core and surface antigens (HBVc, HBVs), hepatitis A, B or C antigens, Epstein-Barr virus antigens, CMV antigens, human immunodeficiency virus (HIV) antigens, herpes virus antigens, and human papilloma virus (HPV) antigens; bacterial and mycobacterial antigens (e.g., for TB, leprosy, or the like); other pathogen derived antigens, e.g., Malarial antigens from *Plasmodium sp.*; or other cellular antigens, e.g., tyrosinase, trp-1. Many other antigen types are known and available, and can be presented by the DC of the invention.

Page 16, line 25:

Both naturally occurring, wild type and mutant, nucleic acids, as well as engineered or altered nucleic acids are favorably employed in the context of the present invention. One of skill will recognize many ways of generating alterations in a given nucleic acid sequence, such as a known cancer marker which encodes an antigen of interest. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, recursive sequence recombination and diversity generation methods of nucleotides (such as, e.g., DNA shuffling), chemical synthesis of a desired oligonucleotide (e.g., in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. See, e.g., Giliman and Smith (1979) Gene 8:81; Roberts et al. (1987) Nature 328:731; Stemmer (1994) Proc Natl Acad Sci U.S.A. 91:10747; Mullis et al. (1987) U.S. Patent No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) and Sambrook, Ausubel, and Berger (*all supra*).

Page 24, line 33 through page 25, line 6.

Most preferably, cells are isolated and characterized by flow cytometry methods such as fluorescence activated cell sorter (FACS) analysis. A wide variety of flow-cytometry methods are known. For a general overview of fluorescence activated flow cytometry see, for example, Abbas et al. (1991) Cellular and Molecular Immunology, W.B. Saunders Company; and Kuby (1992) Immunology, W.H. Freeman and Company, as well as other references cited above, e.g., Coligan. Fluorescence activated cell scanning and sorting devices are available from e.g., Becton Dickinson, Coulter.

Page 33, lines 25 through 31:

The present invention provides mononuclear cell- or monocyte-derived APC and DC subsets (or subtypes) exhibiting phenotypically and functionally novel properties, features, and characteristics. For clarity and to distinguish these novel dendritic cells from conventional DC, DC of the present invention exhibiting the characteristics, features and properties described herein are termed "mDC2," or dendritic cells (DC) of the present invention. Conventional DC exhibiting commonly known characteristics, features and properties are termed "mDC1" or conventional DC.

Page 34, lines 28 through 32:

The mDC2 of the present invention are further distinguished from mDC1 by their cytokine production profile. MDC2 secrete increased levels of IL-10 compared with mDC1. Additionally, mDC2 produce no IL-12 upon activation with LPS plus IFN-γ or anti-CD40 mAbs, LPS plus IFN-γ, whereas conventional mDC1 cells produce high levels of IL-12 when activated under identical culture conditions.

Page 35, lines 11 through 32:

The mechanisms initiating Th2 cell differentiation have been intensely investigated, because professional APCs, such as DC, are known to produce large quantities of IL-12, the most potent cytokine directing Th1 response. The underlying mechanisms mediating Th2 cytokines IL-4 and IL-13 dominate in certain disease situations, such as allergy resulting in increased IgE production (Punnonen et al. (1993) Proc Natl Acad Sci USA 90:3730; Punnonen et al (1998), in Allergy and Allergic Diseases: The New Mechanisms and Therapeutics (J.

Denburg ed. Humana Press, Totowa, p.13). IL-4 is well known to efficiently direct Th2 responses, but no IL-4 production has been demonstrated by professional APCs. $NK1.1^+$ T cells, a numerically minor T cell subset, have been shown to produce high levels of IL-4 and are likely to contribute to the initiation of Th2 response (Yoshimoto et al. (1995) Science 270:1845). However, they are not likely to be the only explanation, because APC typically secrete high levels of IL-12. It was recently shown that plasmacytoid cell-derived DC produce low levels of IL-12 and direct Th2 differentiation, whereas monocyte-derived DC produce high levels of IL-12 and skew T cell differentiation towards Th1 (Rissoan et al. (1999) Science 283:1183), indicating that APCs do differ in their capacity to produce cytokines. Importantly, however, two different cell populations were used as the starting material to generate these subsets, and it remained unclear whether one population has the capacity to differentiate DC subsets with different cytokine production profiles and capacities to mediate Th cell differentiation (Rissoan, supra; Bottomly (1999) Science 283:1124). With results described herein and the mDC2 of the present invention demonstrate that PB monoctyes can differentiate into at least two different subsets that differ from each other in cytokine synthesis profile, surface marker expression and capacity to direct Th differentiation.

Page 36, lines 7 through 12:

In contrast to mDC1, mDC2 do not mature into $CD83^+$ DC in the presence of LPS plus IFN-γ, indicating the signaling requirements for maturation between these two DC subsets are not identical. In addition, because mDC1 molecules can act as efficient lipid antigen-presenting molecules (Beckman et al. (1994) Nature 372:691; Sugita et al. (1999) Immunity 11:743), the fact that mDC2 remain $CD1a^-$ upon maturation further supports the belief that the mDC2 subset is phenotypically and functionally distinct from the mDC1 subset.

Page 37, line 31 through page 38, line 7:

Also included are compositions comprising APC and $CD1a^-$ dendritic cells of the invention. The $CD1a^-$ dendritic cells are capable of presenting an antigen to a T cell. Additionally, in such composition $CD1a^-$ dendritic cells may produce substantially no IL-12 and/or promote differentiation of T cells to a Th0/Th2 subtype. In some such compositions, the

$CD1a^-$ dendritic cells display or present at least one antigen or antigenic fragment thereof. In some such compositions, the at least one antigen or antigenic fragment comprises a protein or peptide differentially expressed on a cell selected from the group consisting of a tumor cell, a bacterially-infected cell, a parasitically-infected cell, a virally-infected cell, and a target cell of an autoimmune response. Such compositions may further comprising a pharmaceutically acceptable carrier, which would be well-known to those of ordinary skill in the art. Certain such compositions may be formulated as a vaccine.

Page 47, lines 16 through 24:

Dendritic cell vaccines utilizing the monocyte-derived APC or mDC2 of the present invention are useful for cancer immunotherapies, including in therapeutic and prophylactic treatment regimens for the following cancers: prostate cancer; non-Hodgkin's lymphoma; colon cancer; breast cancer; leukemia; melanoma; brain, lung, colorectal, and pancreatic cancers; renal cell carcinoma; and lung, colorectal, pancreatic B-cell lymphoma, multiple myeloma, prostate carcinomas, sarcomas, and neuroblastomas, including those cancers described in Timmerman et al. (1999) Annu. Rev. Med. 50:507-29. The antigens for such cancers are present in Timmerman et al., id. at 523. Such antigens can be presented or displayed on the APC or mDC2 of the invention (using peptide loading, pulsing or transfection methods described above).

Page 53, line 36 through page 54, line 10:

Relative IL-12 production by DC generated under the culture conditions described above is shown in Figure 1. PB monocytes were cultured in the presence of IL-4 (400 U/ml) and GM-CSF (800 U/ml) in either RPMI (n=15), IMDM (n=4) or Yssel's medium (n=14). In some cultures, IL-6 (100 U/ml) (n=3) or IL-10 (100 U/ml) (n=4) were added at the onset of the cultures, or anti-CD40 mAbs (10 μg/ml) were included on day 5 (n=11) and studied as indicated in the Figure 1. After a culture period of six days, the cells were harvested and activated with LPS (1 ng/ml) plus IFN-γ (10 ng/ml). The supernatants were harvested after culturing for an additional 24 hours, and the levels of IL-12 in the supernatants were measured by ELISA. The results are expressed as mean±SEM.

Page 54, line 14 through 38:

Each of the components of Yssel's medium, namely insulin, transferrin, linoleic acid, oleic acid, and palmitic acid, has been shown to affect the function of lymphoid cells in vitro and/or in vivo (see, e.g., Lernhardt (1990) Biochem. Biophys. Res. Commun. 166:879; Wooten et al. (1993) Cell. Immunol. 152:35; Karsten et al. (1994) J. Cell. Physiol. 161:15; Okamoto et al. (1996) J. Immunol. Meth. 195:7; and Kappel et al. (1998) Scand. J. Immunol. 47:363). To further characterize the culture conditions that favor mDC2 differentiation, we added individual components of Yssel's medium to RPMI, and analyzed IL-12 production and CD1a expression. In addition, because IMDM differs from RPMI in that it contains higher concentrations of glucose, and because glucose has been shown to influence cytokine production by monocytes, with higher glucose concentrations enhancing cytokine production (see, e.g., Morohoshi et al., (1996) "Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro," Diabetes 45:954), we also studied the effect of glucose on differentiation of DC. Addition of glucose at concentrations 4.5 mg/ml and 9.0 mg/ml did not significantly alter or inhibit (n=2) IL-12 production by conventional DC generated in RPMI (compared to DC generated in Yssel's medium), whereas a combination of linoleic acid, oleic acid, and palmitic acid inhibited, but never completely blocked, CD1a expression on mDC1 (data not shown). Nevertheless, under the experimental conditions described herein, no single component of Yssel's medium was able to fully substitute the effect of the complete medium in inducing altered cytokine production in differentiated DC cells (i.e., differentiation of mDC2) (data not shown). Moreover, if the monocyte cultures were initiated with RPMI, and Yssel's medium was added after 24 hours after the onset of the cultures, the cells differentiated into conventional mDC1 producing high levels of IL-12 upon activation (data not shown), demonstrating that DC differentiation into subsets with different cytokine production profiles is dependent on a delicate balance of growth factors that are present during the initial stages of DC differentiation.

Page 55, lines 18 through 27:

No significant difference in the mean fluorescence intensity (MFI) of these antigens was observed irrespective of whether the cells were differentiated in the presence of

RPMI or Yssel's medium. In addition, no differences in the expression levels of CD13, CD23, CD32, CD33, CD54, and MHC class I molecules between these DC populations were observed, and both subsets (subtypes) also expressed CD47 (data not shown). Furthermore, the DC differentiated either in the presence of Yssel's medium or RPMI strongly downregulated expression of CD14 (as an indication of differentiation into DC) (Fig. 2), demonstrating a phenotype of conventional DC. As a control, monocytes differentiated in the presence of M-CSF in either medium differentiated into macrophages expressing high levels of CD14 with macroscopic appearance of macrophages (data not shown).

Page 57, line 25 to page 58, line 6:

The cytokine production profiles of mature mDC1 and mDC2 were essentially the same as those of the corresponding CD83- population subsets. Regarding IL-12 production, supernatants of mature mDC1 contained 2897±937 picogram/milliliter (pg/ml) IL-12 (mean±SEM), whereas those of mDC2 derived from the same donors contained 125±93 pg/ml IL-12 (n=10). Specifically, in 8 out of 10 experiments, IL-12 production from mature mDC2 was undetectable in ELISA assays in which IL-12 sensitivity is 5 pg/ml. The average of mature mDC2 IL-12 production of 10 experiments was 125±93 pg/ml IL-12 (n=10). The term "substantially lacks IL-12 production," "substantially lacking in production of IL-12," "substantially decreased production of IL-12," or "produces substantially no IL-12" in reference to mature mDC2 IL-12 production refers to a substantial decrease or substantial lack in mature mDC2 IL-12 production relative to the mature mDC1 IL-12 production, and typically refers to a mature mDC2 IL-12 production ranging from at least about 50% to about 100% less, at least about 60% to about 100% less, at least about 70% to about 100% less, at least about 80% to about 100% less, at least about 90% to about 100% less, at least about 95% to about 100% less, at least about 97% to about 100% less, or at least about 99% to about 100% less, than mature mDC1 IL-12 production.

Page 58, lines 7 through 19:

Regarding IL-10, IL-10 production was undetectable in cultures of mature mDC1 (using the ELISA assays in which IL-10 sensitivity is 5 pg/ml), whereas 215±23 pg/ml

(mean+SEM) of IL-10 was produced in the supernatants of $CD83^+$ mDC2 (n=4). The term "substantially increased IL-10 production," "substantially increase in production of IL-10," "substantially increased production of IL-10," or "substantially enhanced production of IL-10" in reference to mature mDC2 IL-10 production refers to a substantial increase or substantial enhancement in mature mDC2 IL-10 production relative to the mature mDC1 IL-10 production, and typically refers to a mature mDC2 IL-10 production ranging from at least about 60% to about 100% greater, at least about 70% to about 100% greater, at least about 80% to about 100% greater, at least about 90% to about 100% greater, at least about 95% to about 100% greater, at least about 96% to about 100% greater, at least about 97% to about 99% greater, or at least about 97% to about 98% greater than mature mDC1 IL-10 production.

Page 60, line 25:

EXAMPLE 7. TRANSFECTION EFFICIENCIES OF MDC2 AND MDC1

IN THE CLAIMS:

Please amend claims 1, 2, 5-7, 10, 11, 13, 17-20, 23-25, 27-35, 37, 39-42, 44, 46, 48-50, 52-57, 60-61, 63, 65, and 68 as indicated below without prejudice to subsequent renewal in their original form. Please cancel claims 36, 43, and 69 without prejudice to subsequent renewal in their original form. Please add new claims 70-78 as follows. For ease of reference and convenience, unamended claims are included below in small type.

1. (Amended) A method of producing at least one differentiated antigen presenting cell (APC), the method comprising: culturing a population of peripheral blood or bone marrow mononuclear cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising insulin, transferrin, linoleic acid, oleic acid, and palmitic acid for a sufficient time to produce the at least one differentiated antigen presenting cell.

2. (Amended) The method of claim 1, wherein the at least one differentiated APC comprises a dendritic cell.

5. (Amended) The method of claim 4, wherein the dendritic cell comprises an mDC2.

6. (Amended) The method of claim 2, wherein the dendritic cell comprises a dendritic cell comprising one or more of the following characteristics: expresses substantially less CD1a on its surface, produces substantially less IL-12, produce substantially a increased amount of IL-10, and induces or promotes T cell differentiation to Th0 or Th2 subtype,

as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

7. (Amended) The method of claim 2, wherein the dendritic cell is capable of presenting at least one antigen to a T cell.

10. (Amended) The method of claim 9, further comprising depleting the population of mononuclear cells with immunomagnetic beads.

11. (Amended) The method of claim 1, further comprising deriving the population of mononuclear cells by density gradient separation of standard buffy coat preparations of peripheral blood.

13. (Amended) The method of claim 12, further comprising depleting the population of mononuclear cells with immunomagnetic beads.

17. (Amended) The method of claim 15, wherein the differentiated APC comprises a dendritic cell.

18. (Amended) The method of claim 17, wherein the dendritic cell substantially lacks IL-12 production compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

19. (Amended) The method of claim 17, wherein the dendritic cell has

substantially increased IL-10 production as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

20. (Amended) The method of claim 2 or 17, wherein the dendritic cell induces or promotes Th0 and/or Th2 differentiation of T cells or substantially lacks expression of CD1a cell surface marker.

23. (Amended) The method of claim 21, wherein the differentiated APC comprises a dendritic cell.

24. (Amended) The method of claim 23, wherein the dendritic cell comprises a dendritic cell that substantially lacks expression of CD1a cell surface marker.

25. (Amended) The method of claim 23, wherein the dendritic cell substantially lacks IL-12 production or induces or promotes differentiation of T cells to Th0/Th2, as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

27. (Amended) The method of claim 23, wherein the dendritic cell comprises one or more of the following characteristics: substantially lacks expression of CD1a cell surface marker, substantially lacks IL-12 production, exhibits increased IL-10 production, and induces or promotes Th0/Th2 differentiation of T cells, as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

28. (Amended) The method of claim 21, further comprising culturing the APC in the presence of an anti-CD40 monoclonal antibody for a period of approximately 24 hours, thereby providing an activated APC; and culturing the activated APC in the presence of lipopolysaccharide (LPS) and interferon-gamma (IFN-g) for a period of approximately 48 hours, thereby producing a mature $CD83^+$ dendritic cell.

29. (Amended) The method of claim 28, wherein the $CD83^+$ dendritic cell comprises one or more of the following characteristics: substantially lacks production of IL-12, exhibits increased IL-10 production, substantially lacks expression of CD1a cell surface marker, and induces or promotes Th0 or Th2 differentiation of T cell, as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

30. (Amended) The method of claim 2 or 24, further comprising introducing to at least one dendritic cell at least one exogenous DNA sequence operably linked to a promoter that is capable of controlling expression of said DNA sequence, which at least one exogenous DNA sequence encodes at least one antigen, in an amount sufficient that expression and presentation of the at least one antigen results, thereby producing an antigen presenting dendritic cell.

31. (Amended) The method of claim 30, further comprising introducing said at least one exogenous DNA sequence to at least one dendritic cell by a method selected from electroporation, injection, microinjection, gene gun delivery, lipofection, DOTAP supplemented lipofection, DOSPER supplemented lipofection, or Superfection.

32. (Amended) The method of claim 2 or 24, further comprising introducing a sufficient amount of at least one antigen or antigenic fragment thereof to at least one dendritic cell, such that presentation of the at least one antigen on least one dendritic cell occurs, thereby producing an antigen presenting dendritic cell.

33. (Amended) A differentiated antigen presenting cell (APC), which differentiated APC expresses substantially less CD1a cell surface marker than a conventional dendritic cell.

34. (Amended) The differentiated APC of claim 33, wherein said differentiated APC comprises a monocyte-derived dendritic cell.

35. (Amended) The differentiated APC of claim 34, wherein monocyte-derived dendritic cell comprises one or more of the following characteristics: substantially lacks IL-12 production, induces or promotes Th0 or Th2 T cell differentiation, and exhibits increased IL-10 production, as compared to a conventional dendritic cell.

Please cancel claim 36 without prejudice to subsequent renewal.

37. (Amended) The differentiated APC of claim 34, wherein the monocyte-derived dendritic cell is produced by culturing a population of monocytes in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid.

39. (Amended) The differentiated APC of claim 37, wherein the monocyte-derived dendritic cell comprises one or more of the following characteristics: substantially lacks IL-12 production, induces or promotes Th0 or Th2 T cell differentiation, substantially lacks CD1a surface marker expression, and exhibits substantially increased IL-10 production, as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

40. (Amended) The differentiated APC of claim 37, wherein the monocyte-derived dendritic cell comprises an mDC2.

41. (Amended) The differentiated APC of claim 37, wherein the monocyte-derived dendritic cell has a transfection efficiency greater than that of a dendritic cell produced by culturing a population of monocytes in IL-4, GM-CSF, and a culture medium comprising RPMI.

42. (Amended) A method of inducing in a subject an immune response to at least one antigen, said method comprising administering to the subject a population of dendritic cells, said dendritic cells displaying or presenting at least one of said at least one antigen, in an

amount sufficient to induce the immune response to said at least one antigen, said dendritic cells comprising one or more of the following characteristics: substantially lacking IL-12 production, inducing or promoting T cell differentiation to a Th0 or Th2 subtype, substantially lacking CD1a surface marker expression, and substantially increasing IL-10 production, as compared to a conventional dendritic cell.

Please cancel claim 43 without prejudice to subsequent renewal.

44. (Amended) The method of claim 42, wherein said dendritic cell is produced by culturing a population of peripheral blood or bone marrow mononuclear cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising insulin, transferrin, linoleic acid, oleic acid, palmitic acid for a sufficient time to produce the differentiated antigen presenting cell.

46. (Amended) A method of inducing differentiation of T cells, the method comprising: co-culturing a population of T cells with a population of antigen presenting cells (APC) that substantially lacks CD1a expression, thereby inducing or promoting differentiation of said T cells.

48. (Amended) The method of claim 46, wherein the antigen presenting cells that substantially lack CD1a expression comprise dendritic cells.

49. (Amended) The method of claim 48, wherein the dendritic cells produce substantially no IL-12, as compared to conventional dendritic cells.

50. (Amended) The method of claim 48, wherein the dendritic cells produce substantially no IL-12 compared to dendritic cells produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

52. (Amended) A composition comprising a population of dendritic cells, said dendritic cells comprising at least one of the following characteristics: substantially lacking

interleukin-12 (IL-12) production, substantially lacking CD1a surface marker expression, exhibiting increased IL-10 production, and inducing or promoting T cell differentiation to Th0 or Th2 subtype, as compared to a conventional dendritic cell.

53. (Amended) The composition of claim 52, wherein said dendritic cells are capable of presenting an antigen to a T cell.

54. (Amended) The composition of claim 52, wherein said dendritic cells produce substantially less or no IL-12 and express substantially less CD1a surface marker, as compared to conventional dendritic cells.

55. (Amended) The composition of claim 52, wherein said dendritic cells promote differentiation of T cells to a Th0/Th2 subtype and produce substantially less IL-12, as compared to conventional dendritic cells.

56. (Amended) The composition of claim 52, wherein said dendritic cells display or present at least one antigen or antigenic fragment thereof.

57. (Amended) The composition of claim 56, wherein the at least one antigen or antigenic fragment comprises a protein or peptide derived from a protein or peptide that is differentially expressed on a cell selected from the group consisting of a tumor cell, a bacterially-infected cell, a parasitically-infected cell, a virally-infected cell, and a target cell of an autoimmune response.

60. (Amended) A method of inducing or modulating an immune response in an immunocompromised subject, said method comprising administering to the subject a population of dendritic cells in an amount sufficient to induce or modulate an immune response in the subject, said dendritic cells exhibiting one or more of the following characteristics: substantially lacking interleukin-12 (IL-12) production, substantially lacking expression of CD1a surface marker, exhibiting increased IL-10 production, and inducing or promoting differentiation of T cells to Th0 or Th2 subtype, as compared to conventional dendritic cells.

61. (Amended) An ex vivo method of inducing in a subject a therapeutic or prophylactic immune response against at least one antigen, the method comprising:

a) culturing a population of monocytes obtained from the subject with IL-4, GM-CSF, and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid for a sufficient time to produce a population of dendritic cells;

b) introducing to the population of dendritic cells a sufficient amount of at least one antigen, or a sufficient amount of an exogenous DNA sequence operably linked to a promoter that controls expression of said DNA sequence, said DNA sequence encoding at least one or said at least one antigen, such that the presentation of the antigen on the dendritic cells results; and

c) administering the antigen-presenting dendritic cells to the subject in an amount sufficient to induce a therapeutic or prophylactic immune response against said at least one antigen.

63. (Amended) A method for therapeutically or prophylactically treating a disease in a subject in need of treatment of said disease, the method comprising:

a) culturing a population of monocytes obtained from the subject with IL-4, GM-CSF, and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid for a sufficient time to produce a population of dendritic cells;

b) introducing to the population of dendritic cells a sufficient amount of at least one disease-associated antigen, or a sufficient amount of an exogenous DNA sequence operably linked to a promoter that controls expression of said DNA sequence, said DNA sequence encoding at least one of said at least one disease-associated antigen, such that presentation of the disease-associated antigen on the dendritic cells results; and

c) administering a therapeutic or prophylactic amount of the dendritic cells presenting the disease-associated antigen to the subject to treat said disease.

65. (Amended) A method for therapeutically or prophylactically treating a disease in a subject in need of treatment of the disease, the method comprising:

a) culturing a population of monocytes obtained from the subject with IL-4, GM-CSF, and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid for a sufficient time to produce a population of dendritic cells;

b) contacting the population of dendritic cells with a population of diseased cells from a tissue or organ of the subject, thereby inducing presentation of a disease-associated antigen on the dendritic cells; and

c) administering a therapeutic or prophylactic amount of dendritic cells presenting the disease-associated antigen to the subject to treat the disease.

68. (Amended) A monocyte-derived dendritic cell, wherein the dendritic cell comprises one or more of the following characteristics: does not substantially express CD1a cell marker, substantially lacks IL-12 production, exhibits increased IL-10 production, and promotes Th0 and/or Th1 lineage differentiation of T cells.

Please cancel claim 69 without prejudice to subsequent renewal.

Please add new claims 70-78 as follows.

70. (New) A population of monocyte-derived dendritic cells produced by culturing a population of monocyte cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising insulin, transferrin, linoleic acid, oleic acid, and palmitic acid, wherein the monocyte-derived dendritic cells comprising an altered cytokine profile compared to dendritic cells produced by culturing a population of monocyte cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

71. (New) The population of monocyte-derived dendritic cells of claim 69, wherein said monocyte-derived dendritic cells comprise one or more of the following

characteristics: produce substantially less interleukin-12 (IL-12), produce substantially more IL-10, express less CD1a cell surface marker, and induce or promote increased T cell differentiation to Th0 or Th2 subtype, as compared to a population of dendritic cells produced by culturing a population of monocyte cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

72. (New) A population of dendritic cells produced by culturing a population of peripheral blood or bone marrow mononuclear cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and Yssel's culture medium, wherein said dendritic cells exhibit one or more of the following characteristics:
substantially lack interleukin-12 (IL-12) production, express less CD1a cell surface marker, induce or promote increased T cell differentiation to Th0 or Th2 subtype, exhibit substantially increased IL-10 production, as compared to dendritic cells produced by culturing a population of monocyte cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

73. (New) A vaccine composition comprising at least one dendritic cell, wherein said at least one dendritic cell comprises one or more of the following characteristics: produce substantially less interleukin-12 (IL-12), produce substantially more IL-10, express substantially less CD1a cell surface marker, and induce or promote increased T cell differentiation to Th0 or Th2 subtype, as compared to a conventional dendritic cell.

74. (New) The vaccine composition of claim 73, wherein the at least one dendritic cell displays or presents at least one antigen or immunogenic peptide on its surface.

75. (New) The vaccine composition of claim 73, further comprising a pharmaceutically acceptable carrier or an adjuvant.

76. (New) The vaccine composition of claim 73, wherein said vaccine composition is useful for prophylactic or therapeutic treatment of cancer.

77. (New) The method of claim 2, wherein the dendritic cell substantially lacks expression of CD1a cell surface marker.

78. (New) The method of claim 61, wherein the dendritic cells comprise mDC2.

REMARKS

Claims 1-69 are presently pending. Claims 1, 2, 5-7, 10, 11, 13, 17-20, 23-25, 27-35, 37, 39-42, 44, 46, 48-50, 52-57, 60-61, 63, 65, and 68 have been amended without prejudice to subsequent renewal in their original form. Claims 36, 43, and 69 have been cancelled without prejudice to subsequent renewal in their original form. New claims 70-78 have been added.

The specification has been amended to correct several inadvertent typographical errors. The amendments to the specification are fully supported by the specification and claims as filed and/or in the patent applications to which priority is claimed and no new matter has been added.

Figures 2 and 3 have been amended to correct an inadvertent typographical error in which Figure 2 was inadvertently mislabeled as Figure 3, and Figure 3 was inadvertently mislabeled as Figure 2 in the present application. Figures 2 and 3 were properly labeled in the two provisional applications to which the present application claims priority. The amendments to the figures are fully supported by the specification and figures as filed and/or in the patent applications to which priority is claimed and no new matter has been added.

The amendments to the pending claims and the newly added claims are fully supported by the specification and claims as filed and/or in the patent applications to which priority is claimed, as discussed in greater detail below, and no new matter has been added. No amendment was made to any claim for any reason related to any statutory requirement for patentability. Applicants respectfully request that the amendments to the claims and specification and the new claims be entered.

Claim 1 has been amended to recite "a method of producing at least one differentiated antigen presenting cell." Support for this amendment is provided throughout the specification, including at page 3, lines 15-37. Claims 2 and 7 have been similarly amended for consistency. Claim 6 has been amended to recite a dendritic cell comprising one or more characteristics; support for this amendment is provided throughout the specification, including at page 3, lines 16-37; page 6, line 23 to page 7, line 13; and page 29, line 19 to page 30, line 9.

Claim 17 has been amended to provide proper antecedent support. Claim 18 has been amended to include the phrase "compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI." Support for this amendment is provided throughout the specification, including at page 29, line 19 to page 30, line 9.

The claim dependency of claim 19 has been amended. Claim 20 has been amended for clarity and to adjust claim dependency; support for this amendment is provided throughout the specification, including at page 3, lines 20-21 and 32-35. Support for the amendment to claim 24 is provided throughout the specification, including at page 3, lines 32-35.

Claims 25, 27, 29 35, 39, 42, 46, 52, 54, 55, 60, and 68 have been amended to provide that the dendritic cell comprises one or more characteristics. Support for these claims is provided throughout the specification, including at page 3, lines 16-37 and page 29, line 19 to page 30, line 9.

Claims 28 and 30-32 have been amended to correct inadvertent typographical errors. Support for the amendment to claim 33 is provided throughout the specification, including at page 3, lines 32-35. Claims 40 and 41 have been amended to adjust claim dependency and antecedent support. Claims 34, 37, 44, and 56 have been amended for consistency.

Claims 46 and 48 have been amended to provide the dendritic cell substantially lacks CD1a expression; support for these amendments is provided throughout the specification, including at page 3, lines 32-35. Claim 49 has been amended for clarity; support for this amendment is provided throughout the specification, including at page 29, line 19 to page 30, line 9.

Claim 50 has been amended to separate the dependency upon claim 3 and claim 48. As amended, claim 50 depends only upon claim 48. New claim 75, discussed below, has been added to include dependency upon claim 3.

Claim 57 has been amended to recite the composition of claim 56, wherein the at least one antigen or antigenic fragment comprises a protein or peptide derived from a protein or peptide that is differentially expressed on a cell selected from the group consisting of a tumor cell, bacterially-infected cell, parasitically-infected cell, virally-infected cell, and a target cell of

an autoimmune response. This amendment has been made for clarity and to correct an inadvertent typographical error. Support for this amendment is provided throughout the specification, including at page 5, lines 18-20, page 8, lines 32-35, and page 37, line 31 to page 38, line 5. The typographical error in the specification has been similarly corrected.

Claims 61-63 have been amended to delete unnecessary language and correct inadvertent typographical errors.

New claim 70 is directed to a population of monocyte-derived dendritic cells produced by culturing a population of monocyte cells in IL-4, GM-CSF, and a culture medium comprising insulin, transferrin, linoleic acid, oleic acid, and palmitic acid, wherein the monocyte-derived dendritic cells comprise an altered cytokine profile compared to dendritic cells produced by culturing a population of monocyte cells in IL-4, GM-CSF, and a culture medium comprising RPMI. New claim 71, which is dependent on claim 70, specifies that such dendritic cells comprise one or more characteristics. Support for claims 70 and 71 is provided throughout the specification, including at page 3, lines 16-37 and page 29, line 19 to page 30, line 9.

New claim 72 specifies a population of dendritic cells produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and Yssel's culture medium, wherein the dendritic cells exhibit one or more of particular characteristics. Support for claim 72 is provided throughout the specification, including at page, including at page 3, lines 16-37; page 29, line 19 to page 30, line 9; and page 31, line 29 to page 32, line 11.

New claim 73 specifies a vaccine composition comprising at least one dendritic cell of the invention. Support for this claim is provided throughout the specification, including at page, including at page 47, lines 16-30. New claims 74-76, each of which is dependent on claim 73, specify further aspects of the vaccine composition. Support for these claims is provided throughout the specification, including at page 5, lines 21-25; page 47, lines 16-30; and page 49, lines 18-36.

New claim 77, which is dependent on claim 2, specifies that the dendritic cell substantially lacks expression of CD1a cell surface marker. Support for these claims is provided throughout the specification, including at page 3, lines 27-37.

New claim 78, which is dependent on claim 61, further specifies that the dendritic

cells comprise mDC2. Support for this claim is provided throughout the specification, including at page 41, line 35 to page 42, line 17.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this Application, please telephone the undersigned at (510) 337-7871.

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Respectfully submitted,

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